# RNA encapsidation by SV40-derived nanoparticles follows a rapid two-state mechanism

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## Supporting information

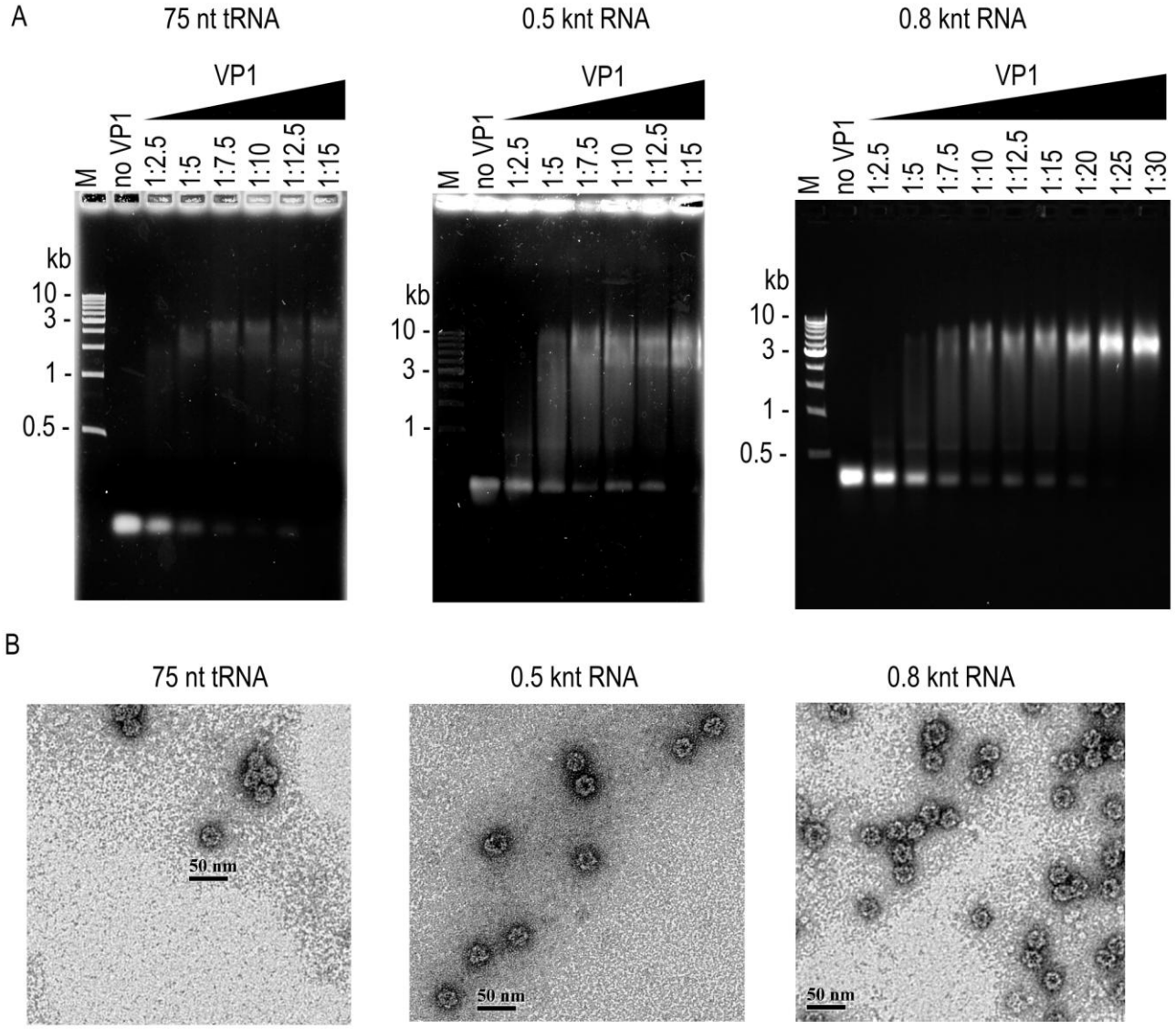


**Figure S1.** Assembly of $VP1_5$ with short RNA molecules. **A.** Titration of short RNAs with VP1 pentamers. Electrophoretic mobility shift assay (EMSA) was analyzed by 0.6% agarose gels. The RNA size and the RNA:VP1 molar ratios are indicated at the top. **B.** Transmission electron microscopy (TEM) images of the corresponding virus-like particles (VLPs) assembly products.

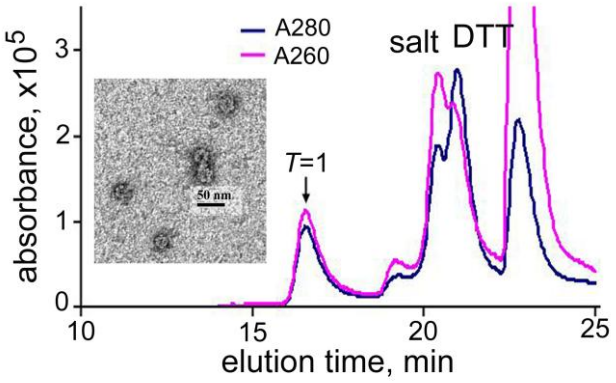


**Figure S2.** Size exclusion chromatography (SEC) of capsids assembled on 75 nt tRNA. The A260/A280 absorbance ratio of 1.19 corresponds to 12 VP1 pentamers per two RNA molecules. Inset: TEM image of the fraction collected from the absorbance peak, showing only 22 nm capsids.

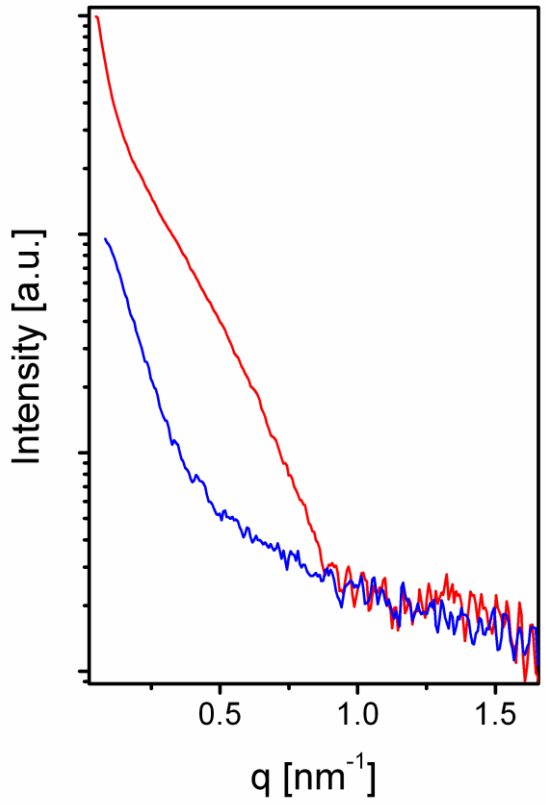


**Figure S3.** Radially integrated SAXS intensities of a solution of 7.5 μM VP1 pentamers (red curve) and a solution of 1 μM 524 nt RNA (Blue curve), measured using the stopped-flow setup.

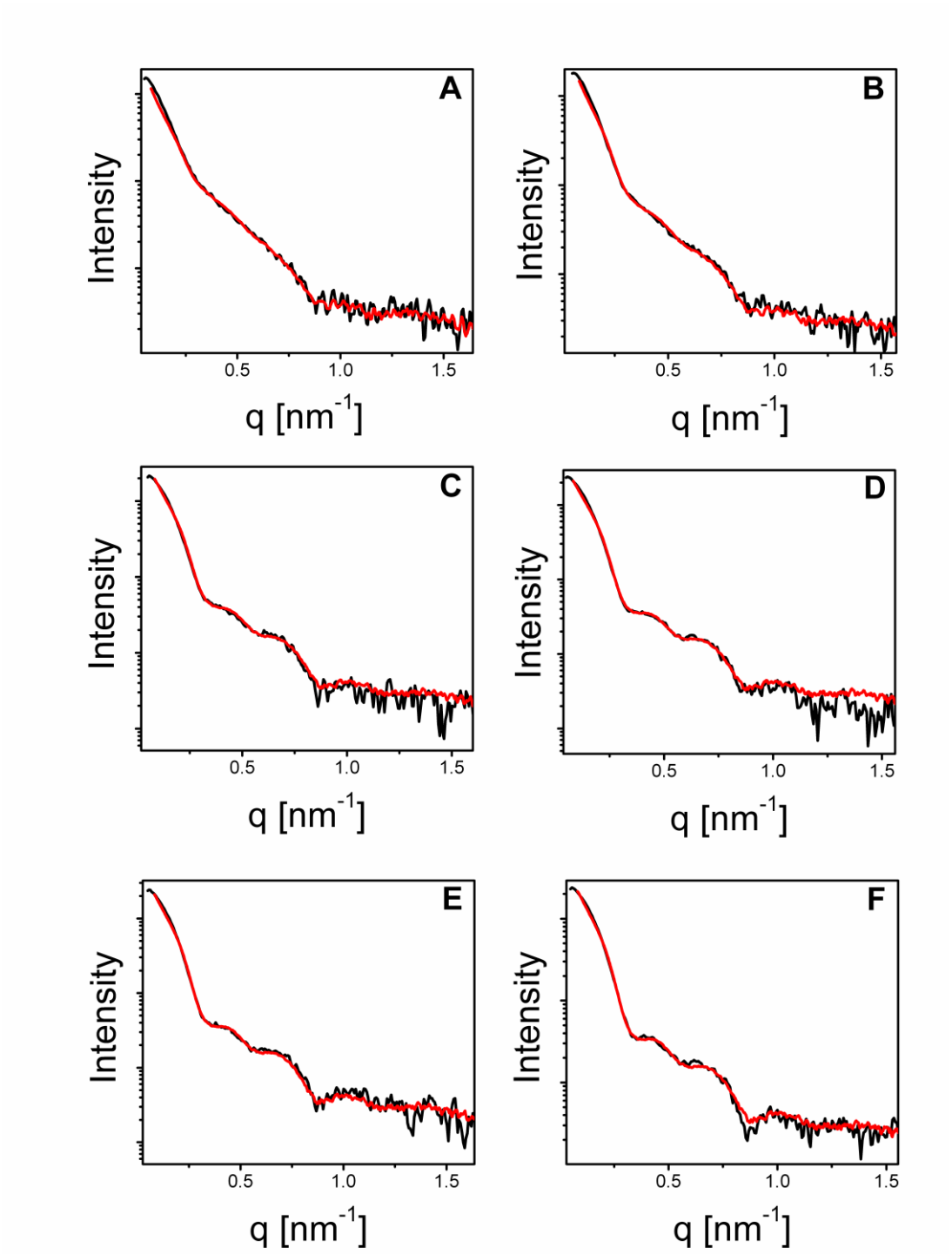


**Figure S4.** Examples of radially integrated time-resolved SAXS (TRSAXS) intensities (black curves) during the assembly process of the (T=1) RNA VLPs and the models (eq 5) that best fitted the data (red curves). The initial concentrations of the 524nt ssRNA and VP1 pentamers were 0.5μM and 7.5μM, respectively. The time, *t*, elapsed after mixing the reactants is: **A.** 0.035sec **B.** 0.085sec **C.** 2sec **D.** 30sec **E.** 40sec **F.** 59sec.

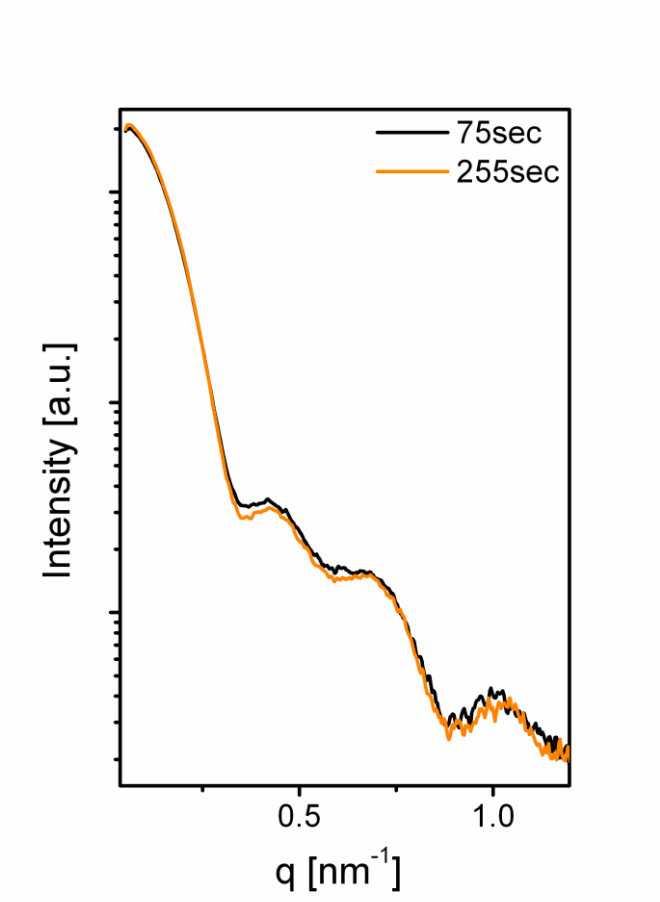


**Figure S5.** Radially integrated TRSAXS intensities measured after mixing equal volumes of 0.6 μM 524 nt RNA with 10 μM VP1 pentamers to form VLPs. The time, *t*, elapsed after mixing the reactants is indicated in the figure. The reaction was measured at the flow-through setup. The exposure time of each measurement was 0.1 sec.

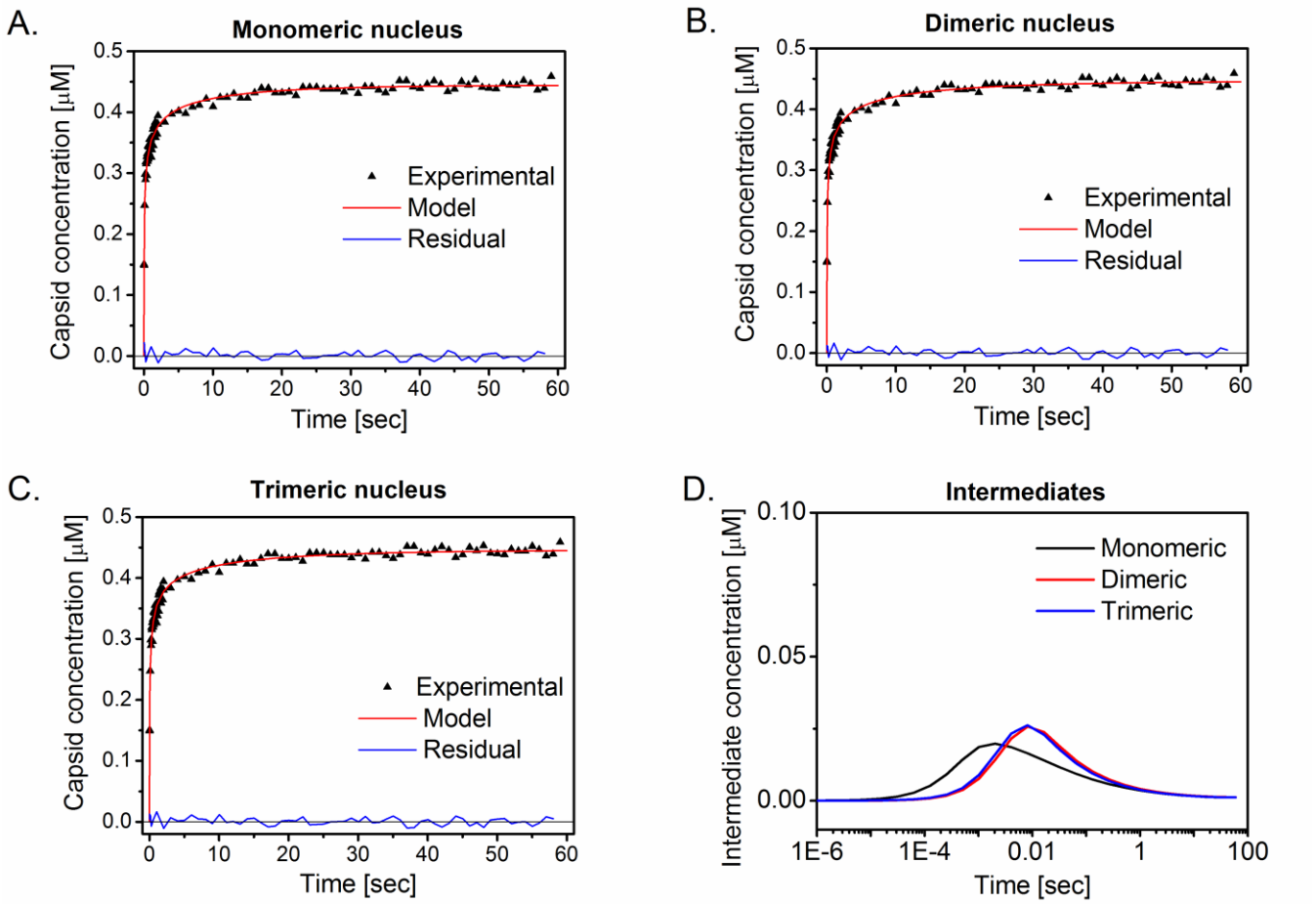


**Figure S6.** Curve fits showing SAXS data (black), the fit curve (red), and residuals (blue). Calculations are for assembly by addition of one pentamer at a time, either in the nucleation or elongation phase of the reaction. All three fits shown closely match the observed kinetics, with no systematic errors obvious in the residuals. The reactions are for assembly of 7.5μM VP1 pentamer with 0.5μM RNA 500mer. The fraction of intermediate structures, obtained in each model, are shown on the right bottom graph.

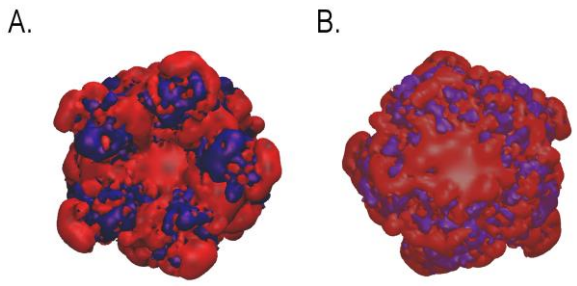


**Figure S7.** Electrostatic Poisson-Boltzmann calculations of the SV40 pentamer [1] calculated via PDB2PQR internet server [2,3] and with the VMD APBS calculator. Red is the isosurface for the potential of *-1* $K_BT$ and blue is for positive potential at *1*$K_BT$ (= 0.0256 eV at room temperature). Inside (**A**) and outside (**B**) view.

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### Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / ‡These authors contributed equally.

## ABBREVIATIONS

VLP, virus-like particles; SAXS, small-angle X-ray scattering; TR-SAXS, time-resolved SAXS; EMSA, electrophoretic mobility shift assay; TEM, transmission electron microscopy.

## REFERENCES


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